CHEMICAL STUDIES ON STREPTOTHRICIN

BY

PAUL KOHN

B.S., Rutgers University, 1941
M.S., University of Illinois, 1948

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
CHEMISTRY IN THE GRADUATE COLLEGE OF
THE UNIVERSITY OF ILLINOIS, 1950

URBANA, ILLINOIS
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INTRODUCTION

Since the discovery that penicillin was a valuable chemotherapeutic agent, there has been intensive research in an effort to find other substances having antibacterial properties which could be used in the treatment of infections. One such antibiotic, designated Antibiotic 20-51 was isolated, and, on characterization, was found to be identical with streptothricin, an antibiotic isolated by Waksman in 1942 (1). A study was then undertaken to determine the chemical nature of this compound.

DISCUSSSION OF RESULTS

Antibiotic 20-51 was isolated from culture filtrates by adsorption on Permutit. The Permutit was washed with water, and the antibiotic eluted by shaking with saturated sodium chloride solution. After evaporation to dryness, the residue was extracted with absolute methanol. Partial purification was achieved by precipitating the active priciple from the methanolic solution by the addition of three volumes of ether. A flaky, white, hygroscopic material was obtained, which was further purified by passage over an alumina column. This resulted in a three fold increase in potency.

A comparison of the physical and chemical properties of Antibiotic 20-51 and streptothricin was made. The results of color tests, infra-red spectra, antibacterial spectra, paper chromatograms of degradation products, and properties of the helianthate salts indicated that these two substances were identical.

Paper chromatography showed that streptothricin was degraded by acid hydrolysis into three components giving positive ninhydrin tests. In the process of hydrolysis there is an increase in amino nitrogen from one out of four nitrogens of the original molecule to three out of four. From 1.66 to 2.10 moles of ammonia per mole of streptothricin are produced, depending on the conditions of hydrolysis. One mole of carbon dioxide is produced per mole of streptothricin. It was found that alkaline hydrolysis caused the evolution of 50 per cent of the carbon dioxide before any ammonia was liberated, and all of the carbon dioxide was evolved when only about 25 per cent of the total amount of ammonia had been liberated. There is no periodate consumption by streptothri-

cin itself, but after hydrolysis one half mole of periodate is taken up per mole of streptothricin.

Extensive studies were made of means to separate the products of hydrolysis of streptothricin. Fractional precipitation with *p*-hydroxyazobenzene-*p'*-sulfonic acid and other sulfonic acids resulted in mixtures which could not be separated readily. The above reagent, however, was useful in fractionating a mixture of two of the ninhydrin positive components of the hydrolysate, and for the preparation of derivatives.

Selective adsorption by ion exchange resins at different pH levels was unsatisfactory for fractionation, but it was found that between pH 8 and 10 the cation exchange resins Amberlite IR-120 and Amberlite IRC-50 adsorbed the degradation products, and at a pH above 10 the anion exchange resin Amberlite IRA-400 adsorbed these substances, indicating the possible presence of negative as well as positive groups in the molecules.

Extraction with organic solvents such as ether, *n*-butanol, phenol and others failed to resolve the mixture.

Chromatographic separations using Solka-floc columns, Celite columns, carbon columns, and strong cation exchange resins were unsuccessful in effecting separation. Alumina chromatography was partially successful, resulting in a small amount of one of the degradation products, but the bulk of it was obtained as a mixture. Conditions suitable for complete separation of one product from the other two were not found.

A satisfactory separation of the three ninhydrin positive components of streptothricin hydrolysates was achieved by chromatography with Amberlite IRC-50. When this resin is buffered at pH 7 the hydrolysis products are retained on the column. By elution with an ammonium chloride-ammonium hydroxide buffer at pH 8.5 the components of the mixture are removed individually.

SUMMARY

An antibiotic was isolated by adsorption with Permutit, elution with saturated sodium chloride and extraction with methanol. Purification was accomplished by alumina chromatography. Comparison of physical and chemical properties of this material with streptothricin showed these two substances to be identical.

Studies were made of the process of degradation of streptothricin. The compounds formed by acid hydrolysis were separated by means of chromatography with Amberlite IRC-50.

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VITA

The author was born in Bayonne, New Jersey, on October 4, 1919. He attended public schools there, and, following graduation from Bayonne High School in June, 1937, he entered Rutgers University, where he held a New Jersey State Scholarship for four years. He received the degree of Bachelor of Science in June, 1941. Until December, 1942, he was employed at the Texas Agricultural Experiment Station, at which time he was inducted into the United States Army. Following his discharge in March, 1946, he was employed by the Best Foods Co. as an analyst. In September, 1947, he entered the Graduate College of the University of Illinois. From September, 1948, to September, 1950, he held an Upjohn Fellowship.

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